

## CLAIMS

1. A method to induce the differentiation of multipotent stem cells by bringing said cells into contact with a pharmacological agent during the process of growth of said multipotent stem cells, wherein said cells are brought into contact with a pharmacological agent in at most four of the growth phases which comprise i) 1<sup>st</sup> development stage, ii) 2<sup>nd</sup> development stage, iii) the first period of 3<sup>rd</sup> development stage, iv) the latter period of 3<sup>rd</sup> development stage, v) the first period of 4<sup>th</sup> development stage, and vi) the latter period of 4<sup>th</sup> development stage, and wherein said agent is a substance which is capable of promoting and/or inhibiting the differentiation of said cells in at least two directions.
2. A method of claim 1 to induce the differentiation of cells wherein the multipotent stem cells are bone marrow stromal cells.
3. A method of claim 2 to induce the differentiation of cells wherein the bone marrow stromal cells have been derived from temperature-sensitive SV-40 T-antigen gene transgenic mice.
4. A method of claim 1 or 2 to induce the differentiation of cells wherein cells are differentiated toward at least two of smooth muscle cells, osteoblasts and adipocytes.
5. A method of anyone of claims 1 to 3 to induce the differentiation of cells wherein the pharmacological agent is cytokine which is capable of promoting or inhibiting the differentiation of cells.
6. A method of claim 4 to induce the differentiation of cells wherein the cytokine is selected from the group consisting of oncostatin M (OSM), bone morphogenetic protein-2 (BMP-2), bone morphogenetic protein-4 (BMP-4), growth differentiation factor 5 (GDF-5) and transforming growth factor (TGF- $\beta$ 2).

7. A method for evaluation of the ability of a pharmacological agent to promote or inhibit the differentiation of cells with use of a method to induce the differentiation of multipotent stem cells by bringing said cells into contact with a pharmacological agent during the process of growth of said cells, wherein said cells are brought into contact with the agent in at most four of the growth phases which comprise i) 1<sup>st</sup> development stage, ii) 2<sup>nd</sup> development stage, iii) the first period of 3<sup>rd</sup> development stage, iv) the latter period of 3<sup>rd</sup> development stage, v) the first period of 4<sup>th</sup> development stage, and vi) the latter period of 4<sup>th</sup> development stage, and wherein said agent is a proposed substance which is expected to promote and/or inhibit the differentiation of said cells in at least two directions.

8. A method for evaluation of claim 7 wherein the multipotent stem cells have been derived from temperature-sensitive SV-40 T-antigen gene transgenic mice.

9. A method for evaluation of claim 7 or 8 wherein the degree of differentiation of the multipotent stem cells which is caused by a proposed agent is to be compared with the degree of differentiation which is caused by bringing said cells, during the process of growth of the cells, into contact with cytokine which is capable of promoting or inhibiting the differentiation of the cells.

10. A method for evaluation of claim 9 wherein the cytokine is selected from the group consisting of oncostatin M (OSM), bone morphogenetic protein-2 (BMP-2), bone morphogenetic protein-4 (BMP-4), growth differentiation factor 5 (GDF-5) and transforming growth factor (TGF- $\beta$ 2).

11. A preparation for regenerative medicine which mainly comprises cells which have been induced by a method of anyone of claims 1, 2 and 4 to 6 to induce cells.

12. A preparation of claim 11 wherein the cells are originated from

mammals.

13. A set of cytokines to regulate the differentiation of cells of mammals, which comprises a combination of two or more cytokines as an effective ingredient, and which is capable of determining three or more directions of differentiation of multipotent stem cells including bone marrow stromal cells, and is capable of regulating the degree of differentiation in each of cells whose differentiation direction has been determined.

14. A set of cytokines of claim 13 wherein the multipotent stem cells are bone marrow stromal cells, and wherein the cells are differentiated in three or more directions.

15. A set of cytokines of claim 14 or 15 wherein the cells are differentiated toward smooth muscle cells, skeletal muscle cells, cardiomyocytes, endothelial cells or adipocytes.

16. A set of cytokines of anyone of claims 13 to 15 wherein the degree of each differentiation is promoted or inhibited by at least 10 % in comparison with the degree of any differentiation which is caused in the presence of serum.

17. A set of cytokines of anyone of claims 13 to 16 wherein the combined two or more cytokines are selected from the group consisting of bone morphogenetic protein-2 (BMP-2), bone morphogenetic protein-4 (BMP-4), oncostatin M (OSM), growth differentiation factor 5 (GDF-5) and transforming growth factor ( $\text{TGF-}\beta 2$ ).

18. A set of cytokines of claim 17 wherein a combination of cytokines is selected from the group consisting of BMP-2 and BMP-4; BMP-2 and OSM; BMP-2 and  $\text{TGF-}\beta 2$ ; BMP-2, BMP-4 and OSM; OSM-BMP-4; OSM and  $\text{TGF-}\beta 2$ ; OSM and GDF-5; OSM, GDF-5 and BMP-4; OSM, GDF-5,  $\text{TGF-}\beta 2$  and BMP-4; BMP-2, OSM, GDF-5 and

BMP-4; and BMP-2, OSM, GDF-5, TGF- $\beta$ 2 and BMP-4.

19. A set of cytokines of anyone of claims 13 to 17 wherein the bone marrow stromal cells are multipotent adult stem cells which can be differentiated toward at least smooth muscle cells, beating cardiomyocytes and endothelial cells with the stimulus of BMP-2.

20. A set of cytokines of anyone of claims 1 to 6 wherein the bone marrow stromal cells have been derived from temperature-sensitive SV-40 T-antigen gene transgenic mice.

21. A set of cytokines of anyone of claims 1 to 8 wherein the differentiation of bone marrow stromal cells is induced in an environment which is selected from the group consisting of *in vitro*, *ex vivo* and *in vivo*.

22. A set of cytokines of anyone of claims 13 to 19 wherein the *ex vivo* or *in vivo* differentiation of bone marrow stromal cells is employed for the transplantation of cells in regenerative medicine.

23. A set of cytokines of claim 20 wherein the *in vitro* differentiation of bone marrow stromal cells is employed for the screening of a pharmacological agent which is capable of differentiating said cells.

24. A method for the screening of medicines which act on the differentiation potency of vertebrate cells, wherein (A) multipotent bone marrow stromal cells derived from temperature-sensitive SV-40 T-antigen gene transgenic mice are prepared, (B) said cells are cultivated in a medium in which the cells may be proliferated in the presence of a proposed agent which is expected to be able to differentiate the cells, (C) the direction or degree of differentiation of thus cultivated cells is determined, and (D) the result of thus determined direction or degree of differentiation is compared with the result of cultivation of said cells in the absence of said agent, and,

then, the difference between said two results is used as an index to the action of said agent on the differentiation potency of bone marrow stromal cells.

25. A method of claim 24 wherein at least two which are selected from the group consisting of BMP-2, BMP-4, OSM, GDF-5 and TGF- $\beta$ 2 are used as comparative agents.

26. A method for screening of claim 25 wherein cell cultivation is conducted in a serum-free medium.